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Bioprocess intensification and optimisation using macroscopic predictive models of cell culture processes

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Bioprocess Intensification and Optimization Using Macroscopic Predictive Models of Cell Culture Processes

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Introduction

Recently, the pharmaceutical industry is increasingly focusing in the use of perfusion mode. Nevertheless, the optimal perfusion rate during biopharmaceutical perfusion production is dependent on cell metabolism which can be characterized by mathematical models. This study provides insights into the predictive capacities of systematic and simple cell modeling approaches of metabolism, growth and production of monoclonal antibodies (mAb) **[1]** to optimize medium composition and perfusion rate during CHO perfusion culture

We applied the metabolic steady state concept and used a segmented linear model to predict cell metabolism. The external metabolite rates are expressed as a linear function of the specific growth rate with various breakpoints associated to metabolic shifts **[1]**. The composition of the perfusion medium was optimized based on this model. This model was used as a controller to determine the daily perfusion rate as a function of the experimental specific growth rate. Two 2 L perfusion cultures using alternative tangential flow (ATF) were performed and analyzed.

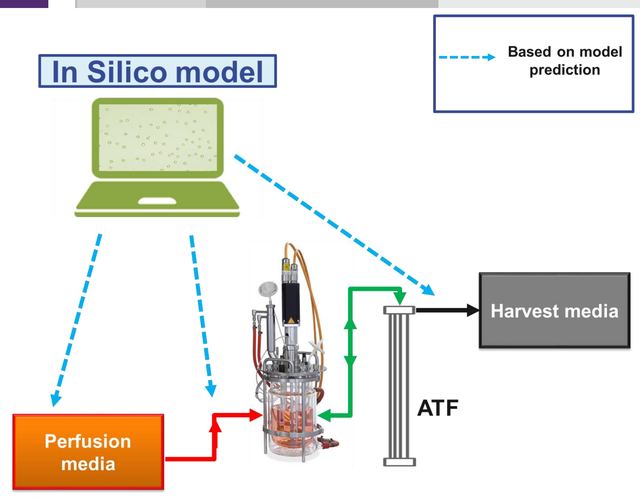

Figure 1 Macroscopic metabolic model application to perfusion production development

Conclusions

- Using the cell metabolism model structure and parameter values from Ben Yahia et al. [1], it was possible to predict metabolic rates of new perfusion cultures in 2 L scale.
- The model was used to determine the medium composition and to control online the daily perfusion rate.

Modeling approach: In Silico model

Metabolic model representation

$$r_i = a_i * \mu + b_i$$

r_i: *specific production rate of metabolite i*

μ: *specific growth rate*

a_i: *specific cell yield on metabolite i*

b_i: *non growth − associated comsumption of metabolite i*

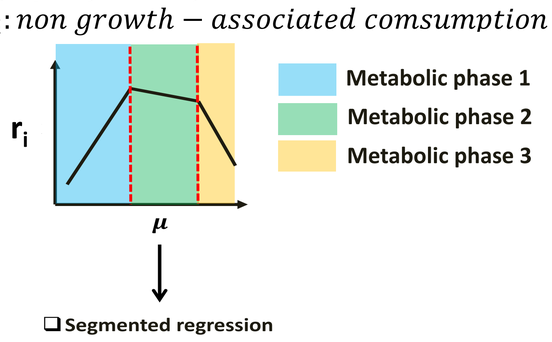

Figure 2 Methodology to predict cell metabolism

Segmented regression is performed on r_i as function of growth rate. Segmented linear regression is a method of regression in which the coefficients of the regression are function of the intervals of the input variable. The model is based on the steady state paradigm.

Model Calibration

The segmented linear model was calibrated with 12*2L bioreactor runs (least-square minimization) in **Fed-Batch mode [1].**

12 bioreactors in fed-batch mode
(model calibration)

Material and Method

The calibrated metabolic model was used to **optimize perfusion media** in order to keep all amino acid constant with one single nutrient source.

Perfusion rate was also adapted for each steady state using calibrated model but also the experimental specific growth rate as an input

Equipments

ATF (Alternate tangential filtration)
- Polyethersulfone fibers, 0.2 µM
- Back-flush
- 0.9 LPM

Culture volume: 1.3L
2 perfusion productions tested with different target viable cell density (VCD) for 20 days.

[1] Ben Yahia, B., Gourevitch, B., Malphettes, L., Heinzle, E., 2016. Segmented linear modeling of CHO fed-batch culture and its application to large scale production. Biotechnol Bioeng. 114(4): 785-797.

Application to Perfusion Productions

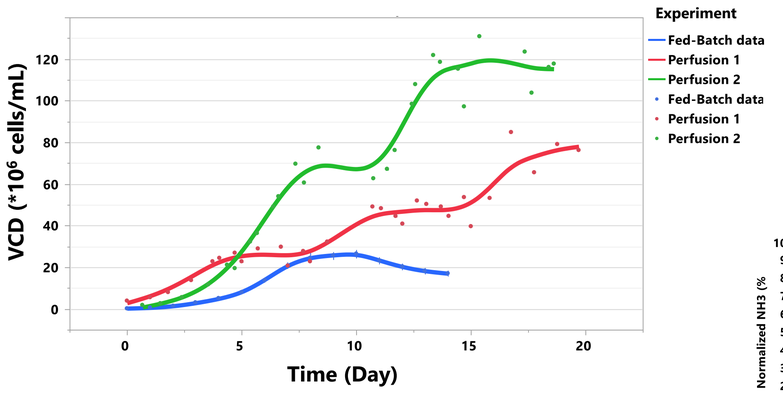

Figure 3

Comparison of the experimental cell growth of 2 perfusion processes with fed-batch process. For "perfusion 1" (red), three VCD were targeted (20, 40 and 70 *10⁶ cells/mL). For "perfusion 2" (green), two VCD were targeted (60 and 120 *10⁶ cells/mL)

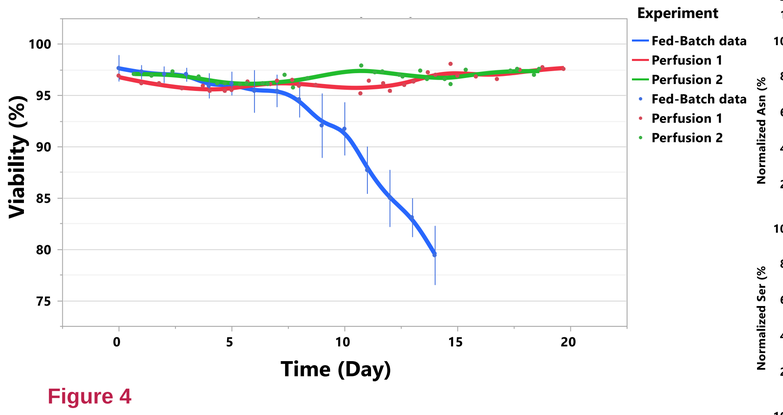

Figure 4

Comparison of the experimental viability of 2 perfusion processes with fed-batch process. For "perfusion 1" (red) and "perfusion 2" (green), viability was maintained constant and higher than 95% during all production whereas viability dropped to 80% for fed-batch process.

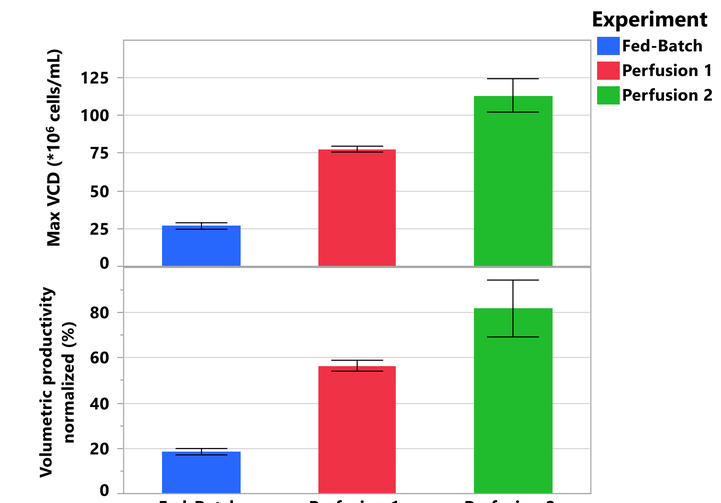

Figure 5

Comparison of the experimental volumetric productivity normalized to the highest volumetric productivity reached. Both perfusion productions reached higher volumetric productivity than fed-batch process. As expected, "perfusion 2" has the higher volumetric productivity which can be related to the high VCD targeted (5 times higher than fed-batch process).

Two perfusion productions were performed using optimized perfusion media and model-based perfusion rates. Data are compared to equivalent fed-batch process.

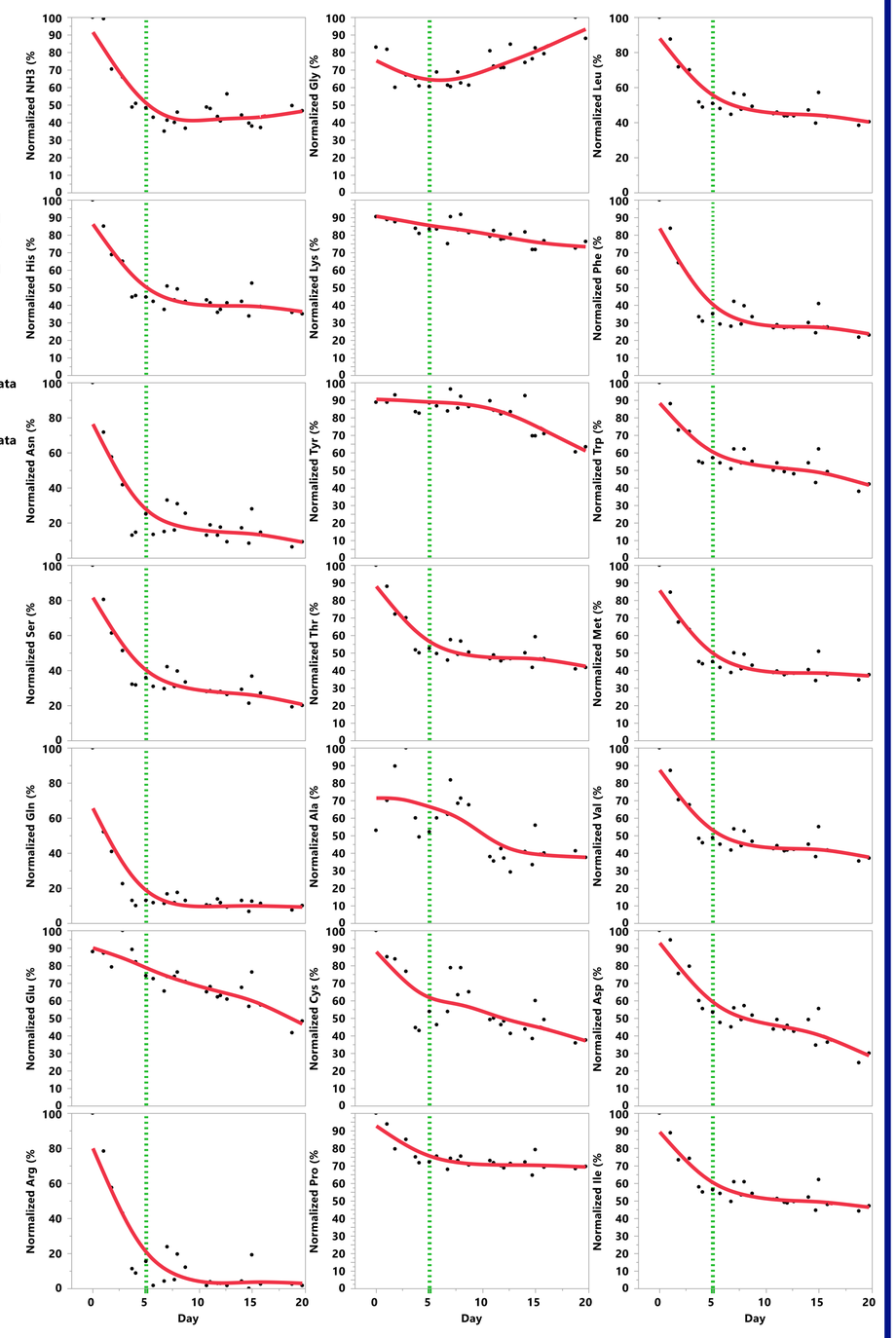

Figure 6

Amino acid concentrations in the bioreactor during "perfusion 1" production. The dashed green line represents when the model was used to calculate the theoretical optimized perfusion rate. For most of the amino acids, the concentration in the bioreactor remains constant after the model was applied. Those profiles support the hypothesis that the model developed for fed-batch production can be applied to perfusion production.

Summary

When traditional experience-based sequential process development is used, the time pressures of the biopharmaceutical industry make it very difficult to fully optimize and understand processes prior to scale-up. Here we showed that applying the model developed in fed-batch by Ben Yahia et al. enables the successful optimization of the perfusion medium composition as well as the online determination of the perfusion rate *in silico*. Hence this novel in silico development approach alleviates the need for labor intensive and time consuming wet experiments while significantly **enhancing performance, accelerating** and **reducing the costs of process development**.

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